

Rapid Invasion Despite Lack of Genetic Variation in the *Erythrina* Gall Wasp (*Quadrastichus erythrinae* Kim)¹

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Abstract: The erythrina gall wasp, *Quadrastichus erythrinae* Kim, has recently and rapidly invaded a broad swath of the tropical and subtropical Pacific Basin, causing severe damage to most species of coral trees (*Erythrina* spp.). This small (length ~1.5 mm) wasp attacks the photosynthetic tissue (leaves, buds, stems, flowers) of ornamental and native *Erythrina*, often killing the trees. This invasion poses an immediate extinction threat to native *Erythrina* spp. throughout Asia, Australia, and a number of Pacific archipelagos, including Hawai'i, where populations of the endemic *E. sandwicensis* have been devastated. Although this pest is known to occur naturally in East Africa, the precise geographic origin of the invasions remains unknown. In this study, 1,623 base pairs of mitochondrial (cytochrome *c* oxidase subunit I) and nuclear DNA (elongation factor alpha) were used to confirm systematic identity and to examine genetic divergence among invasive populations from Hawai'i, Guam, American Samoa, Japan, Singapore, Taiwan, and China. Samples from all invasive populations included in our study showed a complete lack of genetic diversity. Molecular findings confirm that a single species, *Q. erythrinae*, is involved in this dramatic, recent range expansion and that introductions may have been associated with population bottlenecks that have reduced genetic diversity in populations sampled. Although reductions in genetic diversity are generally considered detrimental to fitness, this study provides an example of invasion success despite a lack of detectable genetic variation. The monomorphic genetic pattern observed also suggests that *Q. erythrinae* initially may have been introduced to one location, and this invasive population may have subsequently served as a source for additional secondary invasions by unknown introduction vectors.

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CORAL TREES (*Erythrina* spp., Leguminosae/Fabaceae) are a widespread and diverse genus of trees with approximately 110 species (Mabberly 1987), with a global tropical distribution. Their range includes not only continental endemic species across Africa, Australia, and South America but also endemic species in the islands of Hawai'i, the South Pacific, Micronesia, and the Caribbean, where they are important components of native ecosystems. Some *Erythrina* species have been widely planted as ornamental trees, prized for their dramatic, seasonal flowers and variegated leaves, and other species are important agricultural resources serving as windbreaks, shade trees for crops (macadamia, coffee, cocoa), living fences, and fodder. In the Hawaiian Islands, the endemic coral tree, or wiliwili, *Erythrina sandwicensis*, histori-

cally has been an important component of cultural activities and mythology; lei were made from its flowers and seeds, wood and bark were used for various purposes including canoe building. The wiliwili tree is one of Hawai'i's few deciduous endemic trees and grows to heights of over 13 m, preferring dry forests of the leeward slopes on all of the main islands, from sea level to an elevation of 600 m (Wagner et al. 1990).

Damage to *Erythrina* trees due to an invasive wasp was first documented in 2003, when ornamental trees in the Mascarene Islands and southern Taiwan (Yang et al. 2004) were infested and defoliated by a then-unknown species of eulophid wasp. Within a year, the wasp had spread throughout Taiwan and reached Singapore and by 2005 was documented in Hong Kong and mainland China (Li et al. 2006) and was reported on O'ahu in the Hawaiian Islands in April of that year (Gramling 2005). Within 6 weeks of its arrival in Hawai'i, the wasp had spread over 500 km, throughout the high islands. In 2004 the wasp was described as *Quadrastichus erythrinae* Kim, the erythrina gall wasp (Kim et al. 2004), from specimens collected in Singapore, Mauritius, and Réunion (regions with no native *Erythrina*). In a span of about 2 yr, the erythrina gall wasp had spread across a tropical swath from Hawai'i to India, a distance of more than 12,546 km, much of it across open ocean (Heu et al. 2005, Schmaedick et al. 2006). The erythrina gall wasp has infested and often essentially eliminated most trees of all species of *Erythrina* in those regions, causing a horticultural disaster. In 2006, the erythrina gall wasp moved into the tropical Atlantic, reaching Florida, and appears poised to spread into the Caribbean and South America, where there are large numbers of endemic *Erythrina* species naive to leaf-galling wasps.

The erythrina gall wasp lays eggs in leaves, petioles, young shoots, and stems, forming galls in green plant tissue, completely deforming the trees and threatening the plants' survival. Larvae of this small (1.5 mm), phytophagous wasp hatch within the galls and feed. During severe infestations, even large mature trees are killed.

By killing off spectacular ornamental specimens, agriculturally valuable plantings, and the endemic *E. sandwicensis*, the erythrina gall wasp has already caused substantial impacts to Hawaiian tourism, agriculture, and native ecosystems. This pattern is typical everywhere the wasp has spread.

Before the 2003 invasions, the erythrina gall wasp was unknown, and there were no other gall-forming wasps recorded from any *Erythrina* across the Indian and Pacific oceans, although it is well known that sub-Saharan Africa hosts a diversity of gall wasps on *Erythrina*. Examination of host-plant relationships of *Q. erythrinae* (using 71 different *Erythrina* species) confirms an African origin for the wasp (Messing et al. in press).

Despite the widespread ecological and economic damage caused by invasive species, biological and genetic factors contributing to success of invaders often remain unclear. Although a number of studies investigating levels of genetic polymorphism in invasive populations have demonstrated decreases in genetic diversity relative to native populations (Colautti et al. 2005, Lindholm et al. 2005, Chandler et al. 2008), there are also many examples where population genetic diversity was not substantially decreased (Holland 2001, Hassan et al. 2003) or, despite decreases, invasions proceeded apparently undeterred by any perceivable decrease in fitness (Tsutsui and Suarez 2003, Le Roux et al. 2007, Chandler et al. 2008).

A single species of erythrina gall wasp has recently been documented as responsible for this devastating invasion, but systematic identity of the wasp has been based solely on morphology and behavior, and questions regarding the relationships among the populations, the number of species involved, the potential for the presence of cryptic species, the number of introductions, the geographic source region, and the genetic diversity of these rapidly invading populations remain unanswered. Tung et al. (2009) found no genetic variation in a mitochondrial and nuclear gene in 47 individuals of the erythrina gall wasp from Mauritius, where it was first recorded, and populations in Singapore and Taiwan, but this result needs to be confirmed

across the broader geographic range of the invasion and with more extensive sampling to understand the role of genetic diversity in promoting the erythrina gall wasp's explosive invasion.

Using both nuclear and mitochondrial DNA markers, we sequenced samples of the erythrina gall wasp from across much of its recently invaded range in the Pacific Basin, including American Samoa, China, Guam, Hawai'i, Japan, Singapore, and Taiwan to address the following questions: Is the invasive erythrina gall wasp a single species, or have multiple gall wasps with similar ecology caused this invasion? If the invasion is a single species, how many times was it introduced and from how many source populations? Is there evidence for population bottlenecks (i.e., indicated by lack of genetic diversity across populations)? The answers to these questions apply directly to future efforts to control the erythrina gall wasp and to prevent future introductions of additional gall wasp species that may cause additional damage.

MATERIALS AND METHODS

Sampling

We acquired samples of the erythrina gall wasp from much of its invasive range across 7,840 km of the tropical Pacific Basin (Figure 1, Table 1). Outgroups included unidentified erythrina gall wasp species collected in South Africa. Specimens were placed into 95% alcohol in the field and transported to our laboratory at the University of Hawai'i. Superficial morphological examination of wasps was conducted for all specimens. Target gene fragments were selected for their rapid substitution rates and to take advantage of the strengths of both nuclear and mitochondrial markers and avoid potential bias due to use of only a single character partition (Rubinoﬀ and Holland 2005).

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNAs were extracted according to the manufacturer's protocol using QIAGEN

DNeasy nucleic acid extraction kits (QIAGEN, Valencia, California). DNAs were eluted in deionized autoclaved water and stored at -80°C . Polymerase chain reaction (PCR) was performed using a PTC-200 thermocycler (MJ Research, Reno, Nevada). Cytochrome *c* oxidase I (COI) primer sequences used were as follows: gene regions were PCR amplified and sequenced using the following primers: COI, Jerry (5' CAA CAT TTA TTT TGA TTT TTT GG) and Pat (5' ATC CAT TAC ATA TAA TCT GCC ATA); nuclear elongation factor (EF1- α), Oscar (5' GGC CCA AGG AAA TGG GCA AGG G) and Bosie (5' CCG GCG ACG TAA CCA CGA CGC). Both COI and EF1- α primers amplified the target fragment consistently under the following PCR conditions: 2 min at 92°C , 30 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 45 sec, with a final 72°C extension for 7 min. PCR-amplified DNA fragments were purified with QIAquick spin columns (QIAGEN), according to the manufacturer's protocol, then checked via agarose gel electrophoresis. Forward and reverse strands were cycle-sequenced using the PCR primers. ABI Prism DYE Terminator Cycle Sequencing Reaction Kits in a thermal cycler (Perkin-Elmer 9700) were used to generate single-stranded products, and sequences were determined using an automated sequencer (ABI 377, PE Biosystems, Foster City, California).

Phylogeny Reconstruction

In cases where different methods give similar or identical topologies, confidence is increased that the results are representative of the evolutionary history of the sequences composing the data set (Cunningham 1997). Therefore a variety of approaches was used for phylogeny reconstruction, including maximum parsimony (MP), maximum likelihood (ML), and minimum evolution (ME) with various models of molecular evolution (e.g., Jukes-Cantor, uncorrected "P," Tajima-Nei, Kimura 2-parameter, Tamura-Nei, Kimura 3-parameter). Statistical support was assessed with 1,000 bootstrap replicates for ML, MP, and ME methods (Felsenstein 1985). Trees

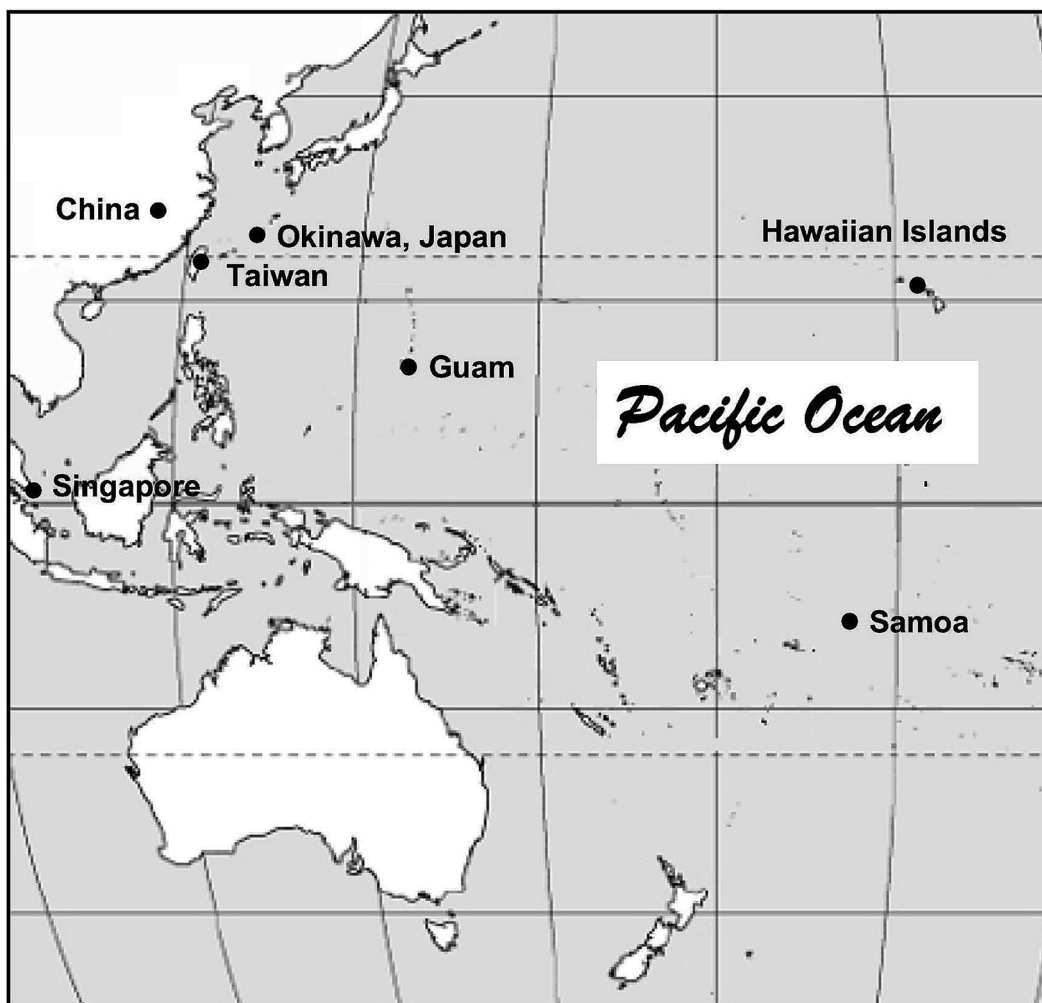


FIGURE 1. Map of the western Pacific Ocean showing sampling localities.

were rooted with multiple outgroups, and resultant topologies were compared for the three approaches (Figures 2 and 3).

RESULTS

Erythrina gall wasp samples from Japan, Taiwan, China, and islands spanning approximately 7,840 km of the Pacific Basin (Figure 1) were monomorphic for both loci sequenced, with a single COI haplotype and a single genotype for the nuclear gene EF1- α

(Figures 2 and 3). After editing and alignment, sequenced gene fragments obtained were 821 base pairs for COI and 802 base pairs for the EF1- α gene. Bootstrap support based on 1,000 replicates for minimum evolution/maximum likelihood/maximum parsimony (ME/ML/MP) was 100% for each optimality criterion. Although we had planned to use statistical parsimony to reconstruct haplotype networks and analysis of molecular variance to elucidate population genetic partitioning, none of these ap-

TABLE 1
Sampling Localities, GeneBank Accession Numbers

Sample Codes (Species, if not <i>Q. erythrinae</i>)	Collection Date	Source	Host Species	Collector	GeneBank Accession No. (COI/EF1- α)
39–47	January 2006	University of Hawai'i at Mānoa campus, O'ahu, Hawai'i	<i>Erythrina</i> spp.	D. Rubinoff	FJ872114/FJ949570
48–52	February 2006	Tutuila, American Samoa	<i>Erythrina variegata</i> <i>orientalis</i>	N. Gurr	FJ872114/FJ949570
54 <i>Quadrastichus</i> sp. 1	June 2006	Somerset West, South Africa	<i>Erythrina</i> sp.	M. Wright, R. Messing, D. Rubinoff	FJ872116/FJ949568
58 <i>Quadrastichus</i> sp. 2	June 2006	Somerset West, South Africa	<i>Erythrina</i> sp.	M. Wright, R. Messing, D. Rubinoff	NA/FJ949569
82 <i>Quadrastichus</i> <i>haitensis</i>	—	Dade County, Homestead, Florida	Pims Palms	R. Duncan	FJ872115/NA
102–106	June 2006	Mangilao, Guam	<i>Erythrina</i> sp.	R. H. Miller	FJ872114/FJ949570
107–112	December 2006	Yozo Itoman City, Okinawa, Japan	<i>Erythrina variegata</i>	N. Uechi (Uechi et al. 2007)	FJ872114/FJ949570
114 <i>Quadrastichus</i> sp. 2	April 2006	Road 40, South Africa	<i>Erythrina latissima</i>	M. Wright, R. Messing, D. Rubinoff	FJ872113/FJ949567
141–146	December 2006	Singapore	<i>Erythrina</i> sp.	R. Messing	FJ872114/FJ949570
TW	—	Taiwan	—	—	EF377343/NA
SY	—	China	—	—	EF377345/NA
SZ	—	Shenzen, Guangdong, China	—	—	EF377346/NA
TW	—	Shenzen, Guangdong, China	—	—	EF377347/NA
ZH	—	Shenzen, Guangdong, China	—	—	EF377348/NA

proaches was appropriate due to a complete lack of polymorphism in all invasive populations sampled.

DISCUSSION

Invasive populations of the erythrina gall wasp have completely defoliated and killed several species of *Erythrina* trees across a wide range of environments. In the Hawaiian Islands, there are some instances where endemic *Erythrina* trees appear to have persisted since the invasion, in spite of infestation, but the trees apparently enter a dormant state

and produce neither leaves nor seeds, effectively reducing (or minimizing) their ecological interactions in the forest.

Destructive insect invasions are well documented and can progress rapidly (MacLeod et al. 2002, Johnson et al. 2006, Muirhead et al. 2006), but the pace and scale of the erythrina gall wasp invasion is unprecedented and represents one of the fastest and most devastating insect invasions ever recorded. Although unintentional anthropogenic introduction is the most likely mechanism driving the explosive spread of the erythrina gall wasp, human-mediated transport has rarely

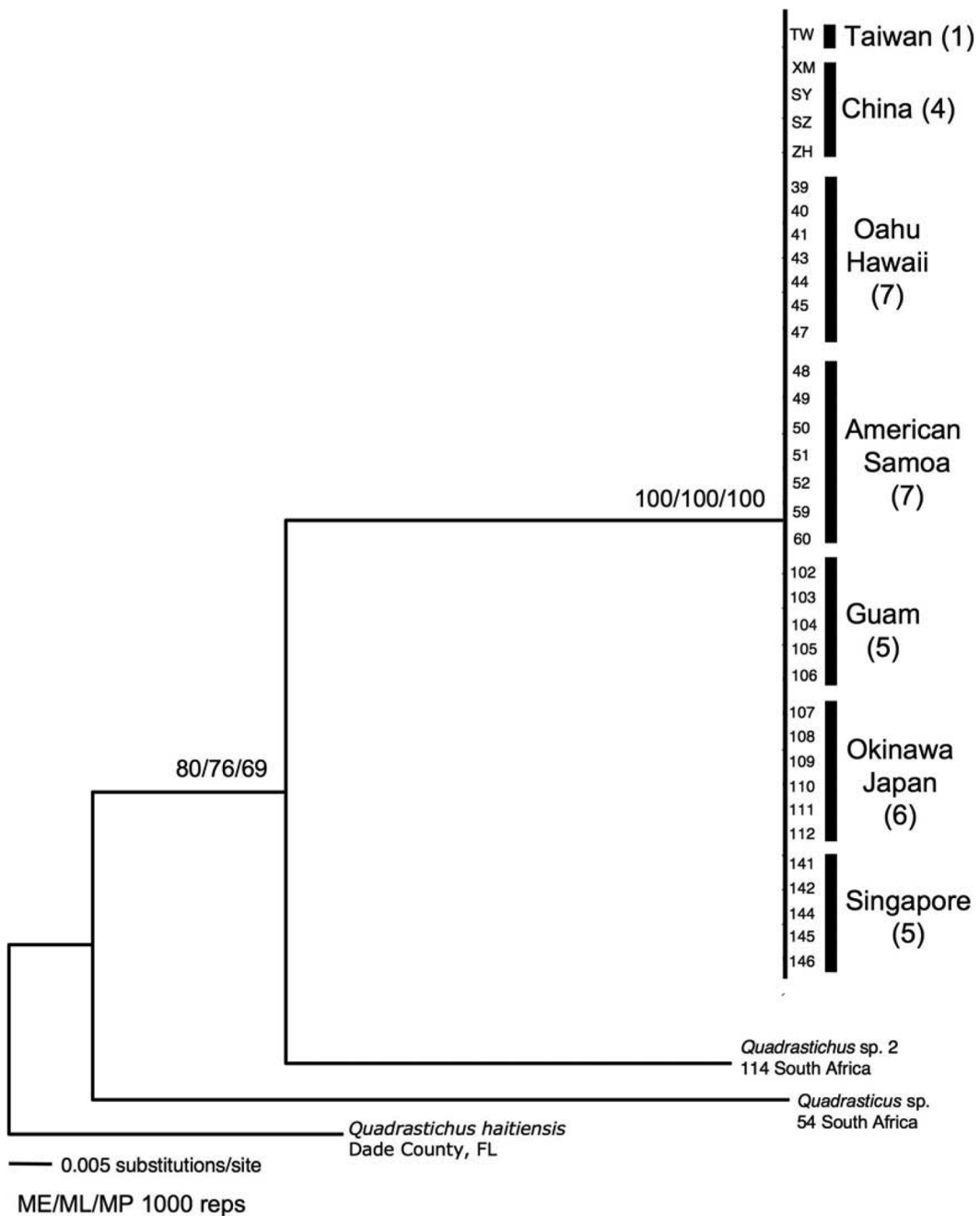


FIGURE 2. Maximum likelihood phylogram with a total of 38 individual wasps, based on 821 base pairs of cytochrome *c* oxidase I (COI). Bootstrap support is shown as follows: minimum evolution/maximum likelihood/maximum parsimony (ME/ML/MP), based on 1,000 replicates for each optimality criterion. Sequence fragments representing 35 invasive wasp specimens (*Quadrastichus erythrinae*) from seven sampling localities shared a single haplotype. Outgroups were collected in South Africa and Florida.

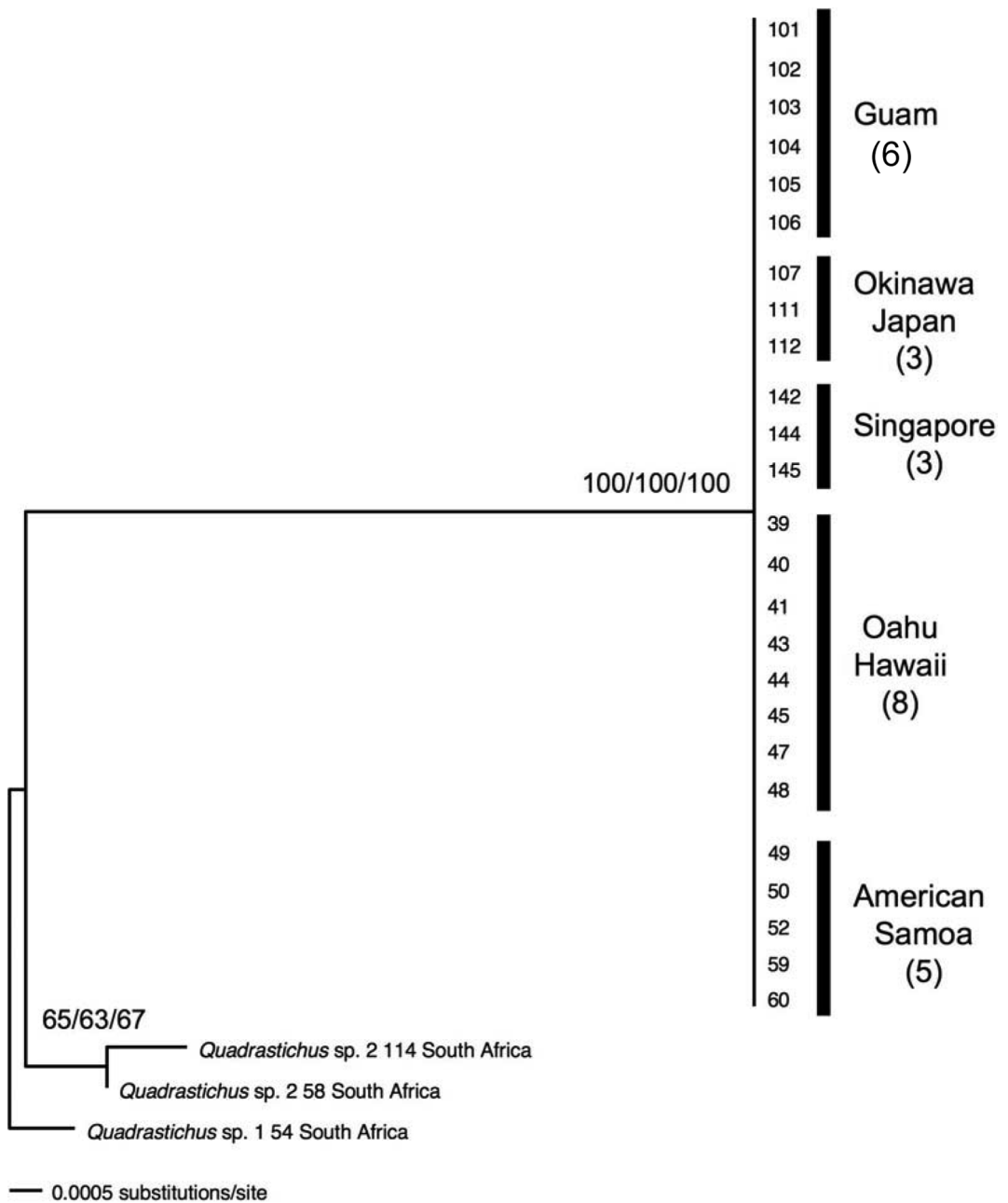


FIGURE 3. Maximum likelihood phylogram based on 802 base pairs of nuclear elongation factor (EF1- α) for 25 invasive Pacific *Quadrastichus erythrinae* plus three outgroups. Note that *Q. erythrinae* nuclear EF1- α from across the Pacific Basin was found to be identical for all ingroup specimens sampled. Bootstrap support shown is based on 1,000 bootstrap replicates as follows: (ME/ML/MP). Outgroups were collected in South Africa.

been so effectively and rapidly exploited by an invasive species.

The erythrina gall wasp invasion represents not only a single widely dispersed species but also a widely dispersed single mtDNA haplotype coupled with no nuclear DNA diversity in a relatively quickly evolving nuclear gene across the Pacific Basin. A complete lack of detectable genetic variation suggests genetic bottlenecks (e.g., Hedrick 2005) and a recent, rapid expansion from a founding population of limited size or at least low genetic diversity. Genetic monomorphism for nuclear and mtDNA gene sequences from all widespread geographic sampling sites suggests that introductions have occurred from a single panmictic population. Although non-native *Q. erythrinae* populations currently appear to be thriving in their new environments, the absence of genetic variation raises questions regarding the long-term persistence of invasive populations (Sakai et al. 2001). Knowledge of the provenance of the erythrina gall wasp's explosive invasion may ultimately be informative in terms of determining the mechanism of transport and release; therefore future research to reveal the geographic source of the invasion will be our focus. As globalization and the speed of commerce and transportation increase, additional potentially invasive species that, like the erythrina gall wasp, until now have gone undetected may exacerbate the pace and level of environmental and economic damage sustained by global ecosystems. However through the application of molecular methods to elucidate taxonomic identity and the geographic source of such invasions (Holland 2000), we gain a powerful tool that sheds light on details of introduction mechanisms that will lead to more effective prediction of impacts and methods of prevention for future invaders.

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